

**TRANSMITTAL OF APPEAL BRIEF**Docket No.  
BGNA046CPRCEIn re Application of: Jeffrey L. BROWNING *et al.*Application No.  
09/626219-Conf. #7978Filing Date  
July 26, 2000Examiner  
M. YuGroup Art Unit  
1642

Invention: Treatment of Follicular Lymphomas Using Inhibitors of the LT Pathway

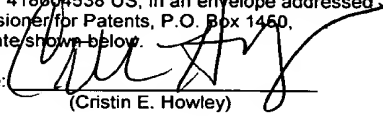
**TO THE COMMISSIONER OF PATENTS:**Transmitted herewith is the Appeal Brief in this application, with respect to the Notice of Appeal  
filed: March 21, 2005The fee for filing this Appeal Brief is \$ 500.00☒ Large Entity ☐ Small Entity☒ A petition for extension of time is also enclosed.The fee for the extension of time is \$ 2,160.00☐ A check in the amount of \_\_\_\_\_ is enclosed.☒ Charge the amount of the fee to Deposit Account No. 12-0080  
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This sheet is submitted in duplicate.Dated: October 21, 2005Cristin E. Howley  
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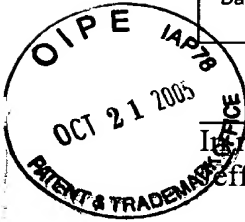
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Dated: October 21, 2005 Signature:   
(Cristin E. Howley)

Docket No.: BGNA046CPRCE  
(PATENT)



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Inter Patent Application of:  
Jeffrey L. Browning *et al.*

Application No.: 09/626219

Confirmation No.: 7978

Filed: July 26, 2000

Art Unit: 1642

For: Treatment of Follicular Lymphomas Using  
Inhibitors of the LT Pathway

Examiner: YU, Misook

**APPEAL BRIEF**

MS Appeal Brief - Patents  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

Appellants file this brief five months more than two months from the filing of the Notice of Appeal filed in this case on March 21, 2005, and in furtherance of said Notice of Appeal.

This appeal brief is accompanied by a petition for the appropriate extension of time for filing this brief and the requisite fees.

This brief contains items under the following headings as required by 37 C.F.R. § 41.37 and M.P.E.P. § 1206:

- I. Real Party in Interest
- II Related Appeals and Interferences
- III. Status of Claims
- IV. Status of Amendments
- V. Summary of Invention
- VI. Grounds of Rejection to be Reviewed on Appeal
- VII. Argument
- VIII. Claims Appendix
- IX. Evidence Appendix
- X. Related Proceedings Appendix

I. Real Party in Interest

The real parties of interest in this appeal are:

Biogen Idec MA (formerly Biogen, Inc.) and New York University

II. Related Appeals, Interferences, and Judicial Proceedings

There are no other appeals, interferences or judicial proceedings known to appellant which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

III. Status of Claims

A. Total Number of Claims in Application

There are twenty-five (25) claims pending in the application.

B. Current Status of Claims

- 1. Claims canceled: 1-19, 21-23, 27-30 and 34-35

2. Claims pending: 20, 24-26, 31-33 and 36-53
3. Claims allowed: none
4. Claims rejected: 20, 24-26, 31-33 and 36-53

C. Claims on Appeal

The claims on appeal are claims 20, 24-26, 31-33 and 36-53.

IV. Status of Amendments

Appellants filed an Amendment After Final on April 11, 2005. Appellants presented no claim amendments in the Amendment After Final. The Examiner issued an Advisory Action mailed August 24, 2005. In the Advisory Action, the Examiner indicated that Appellants' Amendment After Final was not in compliance with 37 CFR 1.121.

Accordingly, the claims enclosed herein as Appendix A reflect the claims as pending.

V. Summary of Invention

The instant invention provides methods and compositions for the treatment of follicular lymphoma tumors which overcome certain problems existing with present therapies, and offers an alternative therapy for those with the specific disorder of follicular lymphoma tumors that are resistant to traditional chemotherapy. In certain embodiments, the claimed invention relates to methods of treating a subject having a follicular lymphoma by administering an effective amount of a composition which includes a soluble lymphotoxin-beta receptor (LT $\beta$ R). Soluble LT $\beta$ R compositions, as featured in the present invention, were well-characterized in the art at the time of filing, and were known to block the interaction of the lymphotoxin- $\alpha/\beta$  (LT-  $\alpha/\beta$ ) heteromer

with its receptor, LT $\beta$ R. Thus, the present invention identifies for the first time the use of such well-characterized, soluble LT $\beta$ R compositions in treatment of the specific disorder of follicular lymphoma. In addition, the present invention also features the use of such compositions for disruption of the interaction of a B cell lymphoma with its environment in a subject.

## VI. Grounds of Rejection to be Reviewed on Appeal

Claims 20, 24-26, 31-33 and 36-51 stand rejected under 35 U.S.C. §112, first paragraph, on the grounds that they fail to comply with the enablement requirement for reasons of record set forth in the previous non-final Office Action of December 22, 2003.

Claims 52-53 stand rejected under 35 U.S.C. §112, first paragraph, on the grounds that they fail to comply with the enablement requirement for reasons of record set forth in the previous final Office Action of September 20, 2004.

## VII. Argument

### Enablement Rejection of Claims 20, 24-26, 31-33 and 36-53 under 35 U.S.C. §112, First Paragraph

The present invention features methods of treatment for the specific diseases of follicular and B-cell lymphomas, *via* administration of an effective amount of a soluble LT $\beta$ R composition to a subject. Soluble LT $\beta$ R is a well-characterized agent known to block the interaction of the lymphotoxin- $\alpha/\beta$  (LT-  $\alpha/\beta$ ) heteromer with its receptor, lymphotoxin-beta receptor (LT $\beta$ R).

The Examiner has rejected claims 20, 24-26, 31-33 and 36-53 under 35 U.S.C. §112, first paragraph, on the grounds that they fail to comply with the enablement requirement. The Examiner contends that “one skilled in the art would have questioned the efficacy of a soluble lymphotoxin beta receptor in treatment of lymphoma since the art as a whole teaches soluble

lymphotoxin beta receptor would make tumor cells grow better rather than killing said cells.” To support this position, the Examiner relies upon the following references: US Patent 5,925,351; Higuchi *et al.* (*Biochem Biophys Res Commun.* 1992 182, 638-43); Qin *et al.* (*Blood*, 1995, 85, 2779-85); Wong *et al.* (*Journal of Cellular Biochemistry*, 1996 60, 56-60); and Reisfeld *et al.* (*Cancer Res*, 1996 56, 1707-12). With regard to Appellants’ results in which soluble LTβR treatment resulted in a five- to ten-fold reduction of lymph node weight in an *in vivo* model of follicular lymphoma, the Examiner states that “[a]s for the lower lymph node weight observed for SJL/RCS mice who received the soluble lymphotoxin beta receptor, one [skilled] in the art might look for explanation elsewhere because Ponzio *et al.* (IDS, 1986, Intern. Rev. Immunol., vol. 1, pages 273-301) at page 288-291 teach unlike other tumor growth, immunosuppression have an adverse effect to effect to transplantability of RCS.” Appellants traverse this rejection of claims 20, 24-26, 31-33 and 36-53 for lack of enablement on two separable grounds: (1) The Examiner has failed to present reason and evidence to doubt the objective truth of Appellants’ statements relied upon for enabling support; and (2) The Examiner has failed to apply the correct legal standard in rejecting Appellants’ claims.

(1) The Examiner Has Failed to Present Reason to Doubt the Objective Truth of Appellants’ Statements Relied Upon for Enabling Support

The standard for establishing therapeutic utility of biotechnological inventions under 35 U.S.C. § 112, first paragraph, was set forth by the Federal Circuit in *In re Brana*, 51 F.3d 1560; 34 U.S.P.Q.2D 1437 (CAFC, decided March 30, 1995). In that case, the Federal Circuit held that a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented, must be taken as in compliance with the enabling requirement of the first paragraph of §112 *unless there is reason to doubt the objective*

*truth of the statements which must be relied on for enabling support.* Thus, the PTO has the initial burden of challenging a presumptively correct assertion of utility. *Only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant* to provide rebuttal evidence sufficient to convince such a person of the invention's asserted utility. See *In re Bundy*, 642 F.2d 430, 433, 209 U.S.P.Q. (BNA) 48, 51 (CCPA 1981).

Here, the Examiner has failed to establish that one of ordinary skill in the art would doubt the objective truth of Appellants' statements related to use of soluble LT $\beta$ R compositions for treatment of follicular lymphoma. Appellants' specification provides extensive teachings and working examples in which soluble LT $\beta$ R compositions were shown to effectively treat tumors in a mouse model of lymphoid tissue tumor (*e.g.*, follicular lymphoma, B-cell lymphoma). As described in the present application, Appellants demonstrated a ***dramatic two- to ten-fold reduction in lymph node weight*** as a result of treatment with soluble LT $\beta$ R compositions in this art-recognized mouse model of lymphoid tumor/follicular lymphoma. Given Appellants' teachings and these dramatic results in an animal model of lymphoid tissue tumor, one of ordinary skill in the art would not have reasonably doubted Appellants' asserted utility.

#### A. The Examiner has Misinterpreted the State of the Art

Appellants respectfully submit that the Examiner's confusion regarding the distinct identities of soluble lymphotoxin (LT $\alpha$ ) and the heteromeric LT- $\alpha/\beta$  (termed "surface LT") molecule featured in the present invention improperly prejudiced the Examiner against Appellants' interpretation of their data. The Examiner's assertion that "one skilled in the art would have questioned the efficacy of a soluble lymphotoxin beta receptor in treatment of lymphoma since the art as a whole teaches soluble lymphotoxin beta receptor would make tumor cells grow better

rather than killing said cells” is based upon art describing the effects of soluble  $LT\alpha$ , a compound that does not signal through  $LT\beta R$ . The Examiner has improperly relied upon references (Higuchi et al., Qin et al., Reisfeld et al. and Wong et al.) addressing the biological role of *soluble  $LT\alpha$*  (also known as  $TNF-\beta$ ), which is involved in a pathway distinct from soluble  $LT\beta R$ .  $LT\alpha$  binds to known  $TNF$  receptors, distinct from  $LT\beta R$ . Independent of this interaction,  $LT\alpha$  forms a surface membrane heteromeric complex with a second molecule called  $LT\beta$ , and *it is the  $LT\alpha\beta$  heteromer complex (also referred to as “surface  $LT$ ”) that binds uniquely to  $LT\beta R$* .  $LT\beta R$  does not bind to secreted  $LT\alpha$ , only to surface  $LT$ . Thus, the surface  $LT$ - $LT\beta R$  complex is a separate system distinct from the interaction of soluble  $LT\alpha$  alone with  $TNF$  receptors. This distinction was well known in the art at the time of filing, and was described in detail in a post-filing date review by Gommerman and Browning (reference of record, *Nature Reviews Immunology*, 2003 3, 642), provided by Appellants to the Examiner during prosecution on the merits to clarify the Examiner’s apparent confusion between soluble  $LT\alpha$  and the  $LT\alpha\beta$  heteromer. In light of the documented distinction that exists between signaling of soluble  $LT\alpha$  and that of the  $LT\alpha\beta$  heteromer complex, the Examiner’s reliance on these references (i.e., Higuchi et al., Qin et al., Reisfeld et al. and Wong et al.) to infer the effect of soluble  $LT\beta R$  treatment as described in the instant invention is improper and without basis in the cited references. As such, the Examiner has failed to present objective evidence that one of ordinary skill in the art would reasonably doubt Appellants’ asserted utility of soluble  $LT\beta R$  compositions for treatment of follicular lymphoma.

The Examiner further contends that the “art as a whole teaches that a soluble lymphotoxin beta receptor inhibits [the]  $LT$  signaling system, which will lead to more cell proliferation, not death.” However, this characterization of the art is also flawed, as the art as a



whole makes no such generalized statement regarding treatments featuring soluble LT $\beta$ R compositions. The Examiner's position derives from combination of a single reference relevant to the activity of soluble LT $\beta$ R (the '351 patent) and four references that describe LT $\alpha$  activity (Higuchi et al., Qin et al., Reisfeld et al. and Wong et al., each of which is irrelevant to LT $\beta$ R signaling for reasons described above). Soluble LT $\beta$ R is a targeted, molecular treatment, the effect of which is known to vary depending upon tissue type and cellular microenvironment. Soluble LT $\beta$ R was shown to inhibit Th1 cell-mediated immune responses in antigen-challenged mice, and to block the apoptotic cell death of a mouse *fibrosarcoma* cell line (refer to the '351 patent). However, soluble LT $\beta$ R treatment can also disrupt lymph node maintenance at a cellular level within the immune system, an effect of soluble LT $\beta$ R that is distinct from that of inducing cell proliferation. In view of the different effects observed for soluble LT $\beta$ R administration in varying tissues and microenvironments, it is incorrect for the Examiner to ascribe a proliferative effect to all soluble LT $\beta$ R treatments. In addition, it is improper for the Examiner to assert that results of the '351 patent regarding soluble LT $\beta$ R treatment of a mouse fibrosarcoma cell line (of mesenchymal origin) will necessarily predict the outcome of soluble LT $\beta$ R treatment of *lymphoid tumors*, as featured in Appellants' present invention. Thus, the Examiner has failed to provide sufficient objective evidence showing that one of ordinary skill in the art would reasonably doubt Appellants' asserted utility of soluble LT $\beta$ R compositions for treatment of follicular lymphoma.

B. The Examiner Presents No Evidence Addressing Use of Soluble LT $\beta$ R Compositions as Treatment for Lymphoid Tumors

In addition to improperly asserting that administration of soluble LT $\beta$ R *must* cause cell proliferation, the Examiner *speculates* that one of skill in the art should necessarily have relied

upon the findings of Ponzio *et al.* (IDS, Intern. Rev. Immunol., 1986 1, 273-301) as teaching that administration of soluble LT $\beta$ R *might* have caused the dramatic reduction in lymph node weight that Appellants observed in an art-recognized model of follicular lymphoma. Setting aside the contradictory nature of the Examiner's assertion that administration of soluble LT $\beta$ R *must* produce cell proliferation, except in those cells in which the Examiner contends it must not (*e.g.*, lymph node cells, in which the Examiner asserts that administered LT $\beta$ R must instead produce a general immunosuppressive effect), the Examiner has cited Ponzio *et al.* based upon the improper assumption that soluble LT $\beta$ R compositions cannot possibly produce a reduction in lymph node size *via* reduction of tumor size (regardless of the significant reduction in lymph node size Appellants have observed). Specifically, the Examiner relies upon Ponzio *et al.* as teaching that immunosuppression hinders the transplantability of RCS. The Examiner concludes from Ponzio *et al.* that "one would conclude that SJL/RCS mice who received the soluble lymphotoxin beta receptor did not have RCS fully transplanted." However, Ponzio *et al.* fails to provide any objective evidence relevant to the presently claimed methods. Ponzio *et al.* describes use of SJL mice to determine the effect of  *$\gamma$ -irradiation and cyclophosphamide* administration on transplantation of spontaneous reticulum cell sarcomas (RCS). Administration of  $\gamma$ -irradiation or cyclophosphamide to SJL mice was found to prevent transplantability of primary RCS, and to diminish the growth of established transplantable RCS lines. Thus, the conclusions of Ponzio *et al.* derive from studies of  $\gamma$ -irradiated or cyclophosphamide-treated SJL/RCS mice. ***Ponzio et al. does not address the effect of a ligand-specific, soluble LT $\beta$ R agent***, and a skilled artisan would not reasonably conclude based on Ponzio *et al.* that the administration of a soluble LT $\beta$ R agent (a much more targeted and subtle treatment than  $\gamma$ -irradiation or cyclophosphamide) would have an inhibitory effect on transplantation *per se*. Indeed, one of ordinary skill in the art would find it

unreasonable to equate the effects of *a whole organism mutagen* such as  $\gamma$ -irradiation or cyclophosphamide with those of a lymphotoxin pathway-targeted, ligand-specific molecular agent such as soluble LT $\beta$ R. Indeed, the Examiner's reliance upon Ponzio *et al.* appears to have resulted from a retrospective analysis performed for the sole purpose of casting doubt upon Appellants' results in an appropriate *in vivo* model of lymphoid tumor. Therefore, the Examiner has failed to assess the patentability of the present invention properly. Simply, *there is no reason based on the evidence relied upon by the Examiner to doubt the objective truth of Appellants' claims. The Examiner has therefore failed to shift the burden to Appellants to provide rebuttal evidence sufficient to convince one of ordinary skill in the art of the invention's asserted utility. Further, one of ordinary skill in the art would not find Appellants' claims to be directed towards an inherently unbelievable undertaking nor to involve implausible scientific principles. Therefore, Appellants submit that the present disclosure fully satisfies the enablement standard set forth in In re Brana, as the present disclosure provides more than a sufficient teaching of how to make and use the claimed methods.*

Finally, the Examiner has improperly relied on post-filing date evidence to show that one of ordinary skill in the art would reasonably doubt Appellants' asserted use of soluble LT $\beta$ R compositions for treatment of follicular lymphomas. The Examiner's reliance on the Gommerman review (introduced by Appellants to clarify the Examiner's confusion of the biological role of soluble LT $\alpha$  and the LT $\alpha/\beta$  heteromer) as objective evidence of what one of ordinary skill in the art would believe regarding Appellants' asserted utility is improper, as this reference was published more than five years after Appellants' priority date. Regardless, it is Appellants' position that the Gommerman review supports Appellants' asserted utility by explaining that the role of soluble LT $\beta$ R is known to vary depending upon tissue type and cellular

microenvironment. This recognition is consistent with Appellants' assertion that soluble LT $\beta$ R compositions can be used to treat follicular lymphoma.

In conclusion, a detailed consideration of the Examiner's evidence fails to establish that one of ordinary skill in the art would doubt the objective truth of Appellants' statements related to use of well-characterized soluble LT $\beta$ R compositions for treatment of the specific, related diseases of follicular and B-cell lymphomas. Appellants emphasize that working examples in the instant specification demonstrate the efficacy of soluble LT $\beta$ R compositions to treat follicular and B cell lymphomas. Appellants have provided working examples in the instant specification that demonstrate the efficacy of soluble LT $\beta$ R treatment in SJL/RCS mice. Appellants have described treatment of mice with soluble LT $\beta$ R as resulting in lower tumor volume in comparison to untreated control mice. Appellants used SJL/RCS mice, an established murine model system used to evaluate tumor treatment, in their featured experiments, as these mice normally develop tumors. Thus, Appellants have demonstrated reduction in tumor volume using soluble LT $\beta$ R in a relevant mouse model through working examples. Quite simply, Appellants have shown that the claimed methods work and provide sufficient guidance for a skilled person to practice the claimed methods. Appellants respectfully submit that nothing more is required.

(2) The Examiner has Failed to Apply the Correct Legal Standard in Rejecting Appellants' Claims

Appellants also submit that the Examiner has improperly required an understanding of the mechanism underlying Appellants' results which has no bearing on the ability of one of ordinary skill in the art to practice the claimed invention. As stated in *Cross v. Iizuka*, "it is axiomatic that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests, nor is the inventor's theory or belief as to how his invention works a necessary element in the specification to satisfy the enablement requirement[.]" *Cross v. Iizuka*,

753 F.2d 1040, 224 USPQ 739 (Fed. Cir. 1985), citing *Fromson v. Advance Offset Plate, Inc.*, 720 F.2d 1565, 1570, 219 USPQ 1137, 1140 (Fed. Cir. 1983). Thus, the disclosure of the precise mechanism of soluble LT $\beta$ R compositions in producing decreased lymph node volume in the SJL/RCS mouse model of follicular lymphoma is not a prerequisite to the patentability of the present invention. Contrary to the holding of *Cross*, the Examiner has required a definitive understanding of the precise mechanism by which soluble LT $\beta$ R compositions cause reduction in tumor size in the art-recognized SJL/RCS model of follicular lymphoma. The Examiner's rejection is therefore improper in view of the legal standard of enablement. The legal standard of enablement requires that a skilled artisan must be able to practice the methods of the invention by treating follicular lymphoma in a subject through administration of a soluble LT $\beta$ R composition of the invention without undue experimentation. A skilled artisan would be able to do so, based on the teachings in Appellants' specification and the knowledge of one skilled in the art.

Appellants submit that the amount of direction or guidance disclosed in the specification is sufficient to enable the skilled artisan to have a reasonable expectation of successfully using the claimed methods without need to perform an undue amount of experimentation. Appellants describe the use of LT $\beta$ R compositions as well as working examples in an *in vivo* model resulting in significantly decreased tumor volume in comparison to untreated control mice. Quite simply, Appellants have shown that the claimed methods work and provide sufficient guidance for a skilled person to practice the claimed methods. Appellants are not legally required to disclose the precise mechanism of soluble LT $\beta$ R activity that caused reduced lymph node weights in the art-recognized follicular lymphoma model employed, yet the Examiner has improperly rejected claims 20, 24-26 and 31-33 and 36-53 for want of such disclosure.

In conclusion, the Examiner has improperly rejected the present claims based upon a misinterpretation of the state of the art as it relates to Appellants' invention. Further, the Examiner has failed to advance objective evidence that one of ordinary skill in the art would reasonably have doubted the presently claimed use of soluble LT $\beta$ R compositions for treating follicular lymphoma in view of Appellants' teachings. Absent such evidence, the present rejection should be withdrawn. Finally, the Examiner has failed to apply the correct legal standard in rejecting Appellants' claims. Appellants have enabled one of ordinary skill in the art to practice the presently claimed invention without undue experimentation.

Accordingly, Appellants believe that they have addressed each of the grounds of the rejection and the rejection has been overcome.

Reversal of the rejection is respectfully requested.

### VIII. Claims Involved in the Appeal

A copy of the claims involved in the present appeal is attached hereto as an Appendix. As indicated above, the claims in the Appendix are the claims as pending.

### IX. Evidence

The Gommerman Review, submitted on June 22, 2004, along with Appellants' Amendment and Remarks.

X. Related Proceedings

No related proceedings are referenced in II. Above, or copies of decisions in related proceedings are not provided, hence no Appendix of Related Proceedings is included.

Appellant believes no fee is due with this statement. However, if a fee is due, please charge our Deposit Account No. 12-0080, under Order No. BGNA046CPRCE from which the undersigned is authorized to draw.

Dated: October 21, 2005

Respectfully submitted,

By 

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**APPENDIX**  
**PENDING CLAIMS**

20. A method for treating follicular lymphoma in a subject comprising administering an amount of a composition comprising a soluble lymphotoxin-beta receptor (LT-beta-R) and a pharmaceutically acceptable carrier, such that treatment occurs.
24. The method of claim 20 wherein the subject is a mammal.
25. The method of claim 24 wherein the subject is a human.
26. The method according to claim 20 wherein the soluble lymphotoxin- $\beta$  receptor comprises a ligand binding domain that can selectively bind to a surface LT ligand.
31. The method of claim 20, further comprising administering to said subject at least one chemotherapeutic agent.
32. The method of claim 20, further comprising administering to said subject a radiation treatment.
33. The method of claim 20 further comprising administering to said subject a radiation treatment or bone marrow transplant.
36. The method of claim 20, wherein the treatment is tumor regression or arrest.



37. The method of claim 20, wherein the soluble LT-beta-R comprises a soluble LT-beta-R fused to one or more heterologous protein domains.
38. The method of claim 37, wherein the soluble LT-beta-R is fused to a human immunoglobulin Fc domain.
39. The method of claim 20, wherein the soluble LT-beta-R comprises an extracellular domain of LT-beta-R.
40. The method of claim 20, wherein the soluble LT-beta-R is human LT-beta-R.
41. The method of claim 40, wherein the soluble LT-beta-R further comprises a human immunoglobulin Fc domain.
42. The method of claim 41, wherein the immunoglobulin is IgG1.
43. A method for disrupting interaction of a B cell lymphoma with its environment in a subject, comprising administering to the subject a composition comprising a soluble LT-beta-R and a pharmaceutically acceptable carrier, such that disruption of the interaction of the B cell lymphoma with its environment occurs.
44. The method of claim 43, wherein the interaction is between the B cell lymphoma and a follicular dendritic cell in the subject.

45. The method of claim 43, wherein the disruption of the interaction results in inhibition of growth of the B cell lymphoma.
46. The method of claim 43, wherein the soluble LT-beta-R comprises a soluble LT-beta-R fused to one or more heterologous protein domains.
47. The method of claim 46, wherein the heterologous protein domain comprises a human immunoglobulin Fc domain.
48. The method of claim 43, wherein the soluble LT-beta-R comprises an extracellular domain of LT-beta-R.
49. The method of claim 43, wherein the soluble LT-beta-R is human LT-beta-R.
50. The method of claim 49, wherein the LT-beta-R-immunoglobulin fusion comprises a human immunoglobulin Fc domain.
51. The method of claim 49, wherein the immunoglobulin is IgG1.
52. A method for treating follicular lymphoma in a human subject, the method comprising administering to the subject a pharmaceutical composition comprising a polypeptide that comprises a soluble, ligand-binding domain of human lymphotoxin-beta receptor (LT-beta-R) fused to a human IgG1 Fc domain, such that treatment occurs.

53. The method of claim 52, wherein the soluble, ligand-binding domain of human LT-beta-R comprises SEQ ID NO:1.

# LYMPHOTOXIN/LIGHT, LYMPHOID MICROENVIRONMENTS AND AUTOIMMUNE DISEASE

Jennifer L. Gommerman and Jeffrey L. Browning

Much of the efficiency of the immune system is attributed to the high degree of spatial and temporal organization in the secondary lymphoid organs. Signalling through the lymphotoxin (LT) pathway is a crucial element in the maintenance of this organized microenvironment. The effect of altering lymphoid microenvironments on immune responses remains relatively unexplored. Inhibitors of the LT and LIGHT pathways have been shown to reduce disease in a wide range of autoimmune models. This approach has provided a tool to probe the effect of manipulation of the microenvironment on both normal and pathological immune responses.

## MARGINAL ZONE

A specialized microenvironment that surrounds the B-cell follicles of the spleen. This compartment is rich in monocytic and dendritic cells that function to capture blood-borne pathogens and present these antigens to both the marginal-zone and memory B cells that reside in this space.

## LYMPHOID ARCHITECTURE

The anatomical framework of the lymphoid organs, including the vascular and lymphatic conduits, extracellular matrix, reticular divisions between various regions and the compartmentalization of cellular subsets.

The immune system is faced with the daunting task of ensuring an encounter between rare antigen-specific T or B cells and minute amounts of pathogen that infiltrate the draining lymph nodes and Peyer's patches or are captured in the MARGINAL ZONE of the spleen. To facilitate these encounters, the immune system has developed highly structured environments in the lymph nodes and the marginal zone of the spleen<sup>1</sup>. Specialized trafficking systems that are orchestrated by components of the addressin, integrin and chemokine families direct the flow of cells into appropriate organs and to sites of inflammation. In the lymphoid organs, the correct positioning of cells is further dictated by the localized production of chemokines and the coordinated expression of various chemokine receptors by the migrating cells depending on their activation and maturation status. These same factors govern the retention or recirculation of lymphoid cells.

During the past 10 years, several observations have led to the realization that the tumour-necrosis factor (TNF) family member lymphotoxin  $\alpha\beta$  (LT $\alpha\beta$ ) is closely linked to the control of LYMPHOID ARCHITECTURE. The genes encoding LT $\alpha$  and TNF were described at the same time, and, indeed, LT $\alpha$  was originally named TNF $\beta$ . Initially, it seemed that LT $\alpha$  was redundant with TNF, as it bound to the same receptors<sup>2</sup>. Only later, with the discovery of the membrane form of LT $\alpha$  in a heteromeric

complex with LT $\beta$  and its unique receptor known as LT $\beta$  receptor (LT $\beta$ R), did a separate biology emerge<sup>3</sup>. The LT system can trigger the development of secondary lymphoid organs, is involved in the formation of ECTOPIC LYMPHOID STRUCTURES and controls aspects of the organization of lymphoid MICROENVIRONMENTS in mature immune systems<sup>4-7</sup>. Attempting to understand how lymphoid microenvironments guide the correct timing and positioning of the various cellular components to mount an efficient immune response is a tremendous undertaking<sup>8</sup>. Analysis of the effects of inhibitors of the LT system in both normal and pathological contexts has provided a new vantage point for understanding how lymphoid microenvironments influence immune responses, and we discuss the potential consequences of altering these lymphoid microenvironments in the context of autoimmune disease.

## Lymphotoxin, LIGHT and their receptors

LT $\alpha$  is a secreted protein, but when co-expressed with a second related protein known as LT $\beta$ , it forms a heteromeric TNF-family ligand that remains tethered to the cell surface through the transmembrane domain of LT $\beta$ <sup>3</sup>. LT $\beta$  does not seem to function by itself; however, the LT $\alpha\beta$  heteromer binds to a TNF-family receptor known as LT $\beta$ R (FIG. 1). The exact role of secreted LT $\alpha$  remains unclear (BOX 1). This system is complicated by

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ECTOPIC LYMPHOID  
STRUCTURES

Organized lymphocytic aggregates that form in sites of chronic inflammation. Typically, T- and B-cell-rich zones are segregated, and dendritic cells (DCs), germinal centres with follicular DC (FDC) networks and specialized endothelia are present. These structures are also known as the 'tertiary immune system' and their formation is termed 'lymphoid neogenesis'.

the existence of a second ligand, known as LIGHT, that binds not only to LT $\beta$ R, but also to an additional receptor known as herpes-virus entry mediator (HVEM)<sup>9</sup>. Cell-surface LT is expressed by activated lymphocytes and a subset of resting B cells, whereas the receptor is expressed mainly by non-haematopoietic and myeloid lineage cells<sup>10–12</sup> (TABLE 1). This pattern indicates that LT, and potentially also LIGHT, functions as a communication link between lymphocytes and stromal cells, but not between T or B cells. The expression patterns of HVEM and LIGHT indicate communication between T cells and dendritic cells (DCs) or T cells and T cells. Given the complexity of these ligand–receptor interactions, we have adapted a nomenclature for this discussion. When LT $\alpha$ -, LT $\beta$ - or LT $\beta$ R-deficient mice or LT $\beta$ -specific antibodies were used to define function, we use the term LT

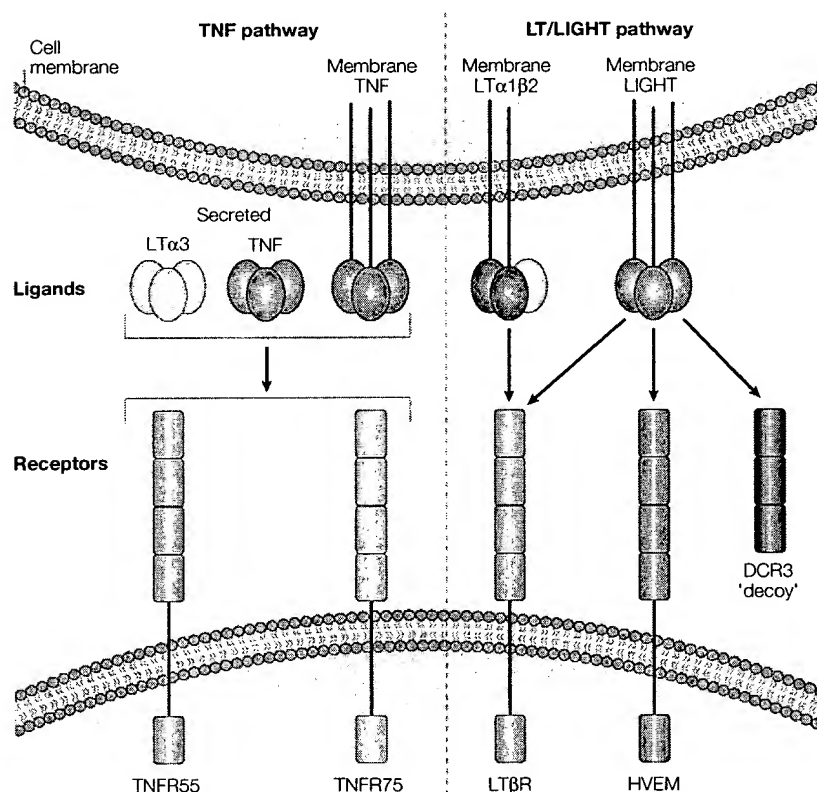
pathway to refer to the membrane LT $\alpha$  $\beta$ -mediated biology. When a decoy receptor, LT $\beta$ R–immunoglobulin fusion protein, was used to block function, LIGHT interactions with either LT $\beta$ R or HVEM cannot be distinguished from the LT pathway and the designation LT/LIGHT pathway is used.

**Lymphotoxin and lymphoid microenvironments**

The seminal discovery of the requirement for LT $\alpha$  for development of the lymph node provided a read-out to distinguish between the functions of LT $\alpha$ , LT $\beta$ , LIGHT and TNF<sup>4,13</sup>. Analysis of genetically deficient mice indicated that LT $\alpha$  $\beta$ –LT $\beta$ R signalling is indispensable for the development of lymph nodes and Peyer's patches. This developmental role of the LT pathway has been extensively reviewed and we limit ourselves here to the functions of this pathway in adult mice<sup>4,5,14–16</sup>. To simplify this review further, we have largely ignored the role of TNF in these processes, even though TNF signalling is clearly involved in several cases. The link between LT and lymphoid microenvironments is well established, yet LIGHT does not share an obvious crucial role with LT in this context<sup>17</sup>. However, the study of LIGHT is still in the initial phase and, as LIGHT can signal through the LT $\beta$ R, we have included this topic. Similarly, because an inhibitor of the dual LT/LIGHT pathway (LT $\beta$ R–immunoglobulin fusion protein) has been used in most of the studies of autoimmune models, the potential for inhibition of LIGHT-mediated events needs consideration. Analysis of LIGHT-knockout mice has uncovered a role in regulating CD8<sup>+</sup> T-cell function and potentially thymic selection<sup>17–19</sup>. LIGHT-transgenic mice develop severe T-cell mediated autoimmune disease and colitis, and these aspects are discussed later<sup>20,21</sup>.

As LT-deficient mice lack most lymph nodes and have impaired development of the spleen, defining the precise role of LT in adult immune physiology and pathology has been problematic. A soluble decoy LT $\beta$ R is an effective inhibitor of both LT- and LIGHT-mediated events and, by comparing the action of this pharmacological agent with the phenotypes of knockout mice and mice that express LT and LIGHT transgenes, the non-developmental roles can be ascertained. A blocking, hamster monoclonal antibody specific for mouse LT $\beta$  has also been successfully used in short-term settings, but antigenicity limits its application in the long term.

The role of LT has been best described in the spleen, yet there are still large gaps in our understanding of its role in lymph nodes and mucosal sites. There is a bewildering array of LT-dependent events (BOX 2) and it is useful to consider a potential general mechanism. One important outcome of the lymph-node development studies was the basic principle that LT-positive lymphocytes communicate with receptor-positive stromal cells to initiate the development of the lymph-node anlagen. In brief, early lymphoid progenitor cells that express LT on the cell surface trigger the expression of LT $\beta$ R by stromal cells, resulting in their maturation, the expression of various adhesion ligands and the secretion of chemokines<sup>4,5</sup>. These chemokines recruit specific cells into the emerging anlagen. Similar LT-induced events



**Figure 1 | Ligands and receptors of the tumour-necrosis factor/lymphotoxin system.**

Two fundamental pathways can be defined. The TNF pathway is activated by tumour-necrosis factor (TNF) or lymphotoxin- $\alpha$ 3 (LT $\alpha$ 3)-induced signalling through the two TNF receptors (TNFRs)<sup>2</sup>. The LT/LIGHT system is composed of a typical TNF family receptor, LT $\beta$  receptor (LT $\beta$ R), that binds to two ligands, the LT $\alpha$  $\beta$  heteromer and homotrimeric LIGHT. LIGHT also binds to two additional receptors in the TNF family, decoy receptor 3 (DCR3) and herpes-virus entry mediator (HVEM). HVEM was originally discovered as the receptor for herpes-virus entry and probably has a role in T-cell function<sup>9</sup>. DCR3 binds effectively to LIGHT, as well as FASL (CD95L) and TNF ligand 1 (TL1, also known as vascular endothelial cell growth inhibitor, VEGI), and because it lacks a transmembrane domain, this soluble decoy receptor might be nature's way of downregulating these pathways<sup>100–102</sup>. Expression of DCR3 is increased in tumours and might be involved in immune escape. The dotted line delineates the two pathways not only on the basis of the different receptor–ligand interactions, but also on divergent biology. LT $\beta$ R signals through both the classic nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway, as well as the alternative NF- $\kappa$ B pathway<sup>103</sup>. Importantly, the alternative NF- $\kappa$ B pathway is only known to be activated by LT $\beta$ R, CD40, BAFFR (B-cell activating factor receptor) and potentially RANK (receptor activator of NF- $\kappa$ B), but not by other receptors, including those for TNF. This aspect of LT $\beta$ R signal transduction begins to provide a molecular explanation for the differences between the LT and TNF pathways<sup>103–105</sup>.

Box 1 | The enigmatic role of soluble lymphotoxin- $\alpha$ 

The role of soluble lymphotoxin- $\alpha$  (LT $\alpha$ ) has perplexed workers in this field. Human LT $\alpha$  is similar in potency to human tumour-necrosis factor (TNF), binds to the TNF receptors (TNFRs) with high affinity and can be detected in the blood of patients with Crohn's disease, T-cell leukaemia and hepatic allograft rejection<sup>2,120–122</sup>. The importance of secreted LT $\alpha$  in humans is unknown. On the basis of concentration, the amounts of LT $\alpha$ 3 in the blood are markedly lower than TNF, although local levels might differ. Antibodies specific for human TNF are an effective therapy in colitis and arthritis without blocking LT $\alpha$ 3, indicating that soluble LT $\alpha$ 3 does not make a crucial contribution to the inflammatory state. The evidence on mouse LT $\alpha$ 3 is more confused, as the available biochemical data indicates that mouse LT $\alpha$  interacts poorly with mouse TNFRs at physiological concentrations<sup>123,124</sup>. Nonetheless, transgenic mice that express LT $\alpha$  develop ectopic lymph-node-like structures, and this phenomenon depends largely on direct signalling of secreted LT $\alpha$ 3 through TNFRs<sup>7,113</sup>. Therefore, similar to human LT $\alpha$ 3, mouse LT $\alpha$ 3 can induce local inflammation and lymphoid neogenesis when it is overexpressed. In these ectopic lymph-node-like structures, formation of complexes with endogenously produced LT $\beta$  — that is, forming cell-surface LT $\alpha\beta$  complexes — is crucial for the development of a specialized endothelium that expresses peripheral lymph-node addressin (PNAD), and the formation of large lymphocytic infiltrates requires the expression of both LT $\alpha$  and LT $\beta$  molecules<sup>6,113</sup>. There are several unresolved differences between LT $\alpha$ - and LT $\beta$ -deficient mice that potentially highlight functions for LT $\alpha$ 3 that are independent from LT $\alpha\beta$ . Notably, there are differences in the presence of mesenteric lymph nodes, the extent of T- and B-cell disorganization and the ability to clear infection with mycobacteria<sup>16,125,126</sup>. Other aspects that could confound the interpretations of these data include possible cross-talk with the TNF pathway by LT $\alpha$ 2 $\beta$ 1, the lack of LT $\beta$  might force the secretion of LT $\alpha$  to supra-physiological levels in knockout mice and potential binding of LT $\alpha$ 3 to herpes-virus entry mediator (HVEM)<sup>127,128</sup>.

are recapitulated in the maintenance of adult lymphoid microenvironments. Here, we consider several of these microenvironments in some detail.

**Follicular dendritic-cell networks.** The follicular DC (FDC) is the best-studied example of the role of LT in the maintenance of reticular cells. This fibroblastoid cell forms a scaffold in the B-cell follicle that is essential for the positioning of B cells, antigen presentation and efficient germinal-centre reactions (BOX 3). LT that is expressed on the cell surface of some B cells functions to maintain FDCs in a fully functional state<sup>22–26</sup>. Whereas the B cell is considered the primary source of LT signalling in the primary LYMPHOID FOLLICLES of the spleen, the source of LT (or LIGHT) signalling in more active settings — such as reactive lymph nodes, GERMINAL CENTRES or even the spleens of recombinase-activating gene (Rag)-deficient mice — is poorly understood<sup>27</sup>. Indeed, the study of a mouse with selective deletion of the gene encoding LT $\beta$  in B cells indicated that non-B cells might also contribute to the maintenance of FDCs in germinal centres of the spleen and lymph nodes<sup>28</sup>. The loss of maintenance signals mediated by LT results in the surprisingly rapid collapse of FOLLICULAR DENDRITIC-CELL NETWORKS<sup>29,30</sup> (FIG. 2). Furthermore, the maintenance of FDCs is required for the constitutive release of CXC chemokine ligand 13 (CXCL13) that functions to recruit B cells into the follicle. So, FDCs attract the cells that are required to maintain their differentiation status<sup>22,23</sup>. This feedback loop is further described in FIG. 2. An additional consequence of the maintenance of FDC maturation is the continued expression of adhesion molecules, such as vascular-cell

adhesion molecule 1 (VCAM1), by the FDC network<sup>30,31</sup>. VCAM1 seems to be a marker for the maturation of FDCs in germinal centres<sup>30,32</sup>. Expression of VCAM1 by FDCs functions to retain B cells in close proximity, and this could be particularly important in the context of a germinal-centre reaction. Expression of VCAM1 and intercellular adhesion molecule 1 (ICAM1) is upregulated by LT $\beta$ R signalling by several fibroblastoid- and epithelial-cell lines<sup>18,31,33</sup>, and VCAM1 expression is a crucial step in the formation of some lymph nodes<sup>4,34</sup>. It is probable that the expression of adhesion molecules by the reticular scaffolds has a fundamental role in lymphoid microenvironments.

**Splenic marginal zone.** Another LT-dependent microenvironment is the marginal zone of the spleen. This specialized compartment has evolved to filter circulating pathogens from the blood and to present them to B cells<sup>35</sup>. The marginal zone is comprised of a reticular matrix embedded with haematopoietic cells, such as metallophilic macrophages, marginal-zone macrophages and DCs. The space contains both memory B cells and a specialized subset of B cells known as marginal-zone B cells. Marginal-zone B cells can respond quickly to antigen and, therefore, are ideally suited to respond to pathogens that are captured from the circulation. Loss of LT signalling either through a genetic deficiency or with an inhibitor in wild-type adult animals results in the loss of various marginal-zone myeloid populations and marginal-zone B cells<sup>16,36–39</sup>. This observation has been made in both rodents and non-human primates — that is, in species with small and large marginal zones, respectively<sup>30,39</sup>. Presumably, an adhesion or chemokine system that depends on stromal- or myeloid-derived LT drives the basic organization of the marginal zone and the retention of the relatively sessile marginal-zone B cells. In addition, marginal-zone B cells are retained in the marginal-zone compartment through interactions with ICAM1 and VCAM1, and the expression of these molecules in the marginal zone is reduced following inhibition of LT<sup>30,36</sup>. In the case of pharmacological inhibition of the LT pathway, the myeloid and marginal-zone B cells disappear more slowly than the collapse of the FDC network. An attractive hypothesis is that LT functions to maintain the maturation state of the marginal-zone reticulum and subsequent organization of haematopoietic cells that are resident in the marginal zone emanates from this scaffold. It is uncertain whether B cells directly provide the relevant signals for marginal-zone maintenance, as selective loss of expression of LT by B cells led only to a partial collapse of marginal-zone integrity<sup>28</sup>.

**T-cell zones.** The development of the reticulum in the T-cell zone of the spleen (BOX 3) depends on LT $\beta$ R signalling during development, but its presence is not altered by the inhibition of LT in adult mice<sup>40</sup>. There is a subset of homeostatic chemokines that dictate the basic positioning in the secondary lymphoid organs — that is, CXCL12, CXCL13, CCL19 and CCL21 (REFS 22,41). Similar to the role of CXCL13 and B-cell positioning (FIG. 2),

## MICROENVIRONMENT

The generic term used to describe the local interplay between mobile lymphocytes and the fixed reticular/stromal cells, and includes cell adhesion, trafficking, chemokine function and cellular positioning.

## LYMPHOID FOLLICLE

A region in organized lymphoid environments that is composed of B cells. Typically, a follicular dendritic-cell (FDC) reticular network marks this region. Germinal-centre reactions occur in this region. The term primary follicle (or mantle in humans) refers to the region that contains follicular B cells that remain outside the germinal centres.

## GERMINAL CENTRE

Also known as a secondary follicle, this highly specialized and dynamic microenvironment occurs in the lymphoid follicles during an immune response. This environment is designed to promote the presentation of unprocessed antigen, the rapid clonal expansion of activated B cells, somatic hypermutation and affinity maturation that culminates in the generation of memory B cells and antibody-secreting plasma cells.

the production of CCL19 and CCL21 at least partially depends on LT signalling in the adult spleen<sup>42</sup>. Several cell types produce these chemokines in the spleen and lymph node, and those that are under the influence of LT are unknown. The deficiency of CCL19 and CCL21 in the *plt* mouse (paucity of lymph-node T cells) and the absence of their receptor in CCR7-deficient mice results in the disruption of naive T-cell entry into the lymph node and altered lymphoid homeostasis<sup>43</sup>. Deletion of the gene encoding LT $\alpha$  or pharmacological disruption of LT function in adult mice leads to a decreased number of DCs in the spleen and impaired positioning of DCs. This observation would be consistent with some alteration in the production of CCL21 by stromal cells in the T-cell zone<sup>27</sup>. So, there seem to be events in the T-cell compartment that are also under the control of LT.

**Mucosal microenvironments.** There are several specialized microenvironments that are designed to coordinate interactions between the intestinal lymphoid system and the commensal bacteria and pathogens in the gut. First, there are the well-organized sites, known as Peyer's patches, in the small bowel that have specialized epithelia dedicated to sensing the gut lumen<sup>44</sup>. Inhibition of LT/LIGHT signalling in adult mice results in flattening of the Peyer's patches, a general reduction in both T- and B-cell numbers in this microenvironment and collapse of the FDC networks<sup>45</sup>. As in the spleen, the number of DCs decrease, especially in the area that underlies the subepithelial dome of the Peyer's patch<sup>45,46</sup>. Inhibition of LT/LIGHT signalling in adult mice also

results in a reduced density of M cells — a population of epithelial cells that sample antigen in the gut lumen<sup>46</sup>.

Second, there are several smaller organized lymphoid aggregates in both the large and small bowel. The large bowel is populated with a structure known as a colonic patch and these seem to increase in number during colitis, indicating that they might not be developmentally predetermined<sup>45,47</sup>. This site is covered by a specialized epithelium that is analogous to the Peyer's patch. Humans have thousands of these patches and it has been suggested that the earliest signs of CROHN'S DISEASE might be seen in these structures<sup>48</sup>. Treatment with LT $\beta$ R-immunoglobulin fusion protein decreases the hypertrophic nature of the colonic patches that are found in colitis induced by trinitrobenzene sulphonate (TNBS)<sup>45</sup>. In the small intestine, there are isolated lymphoid follicles that are positioned in specialized villi and even smaller aggregates in the crypts known as cryptopatches, which contribute to the extrathymic development of T cells<sup>49</sup>. Isolated lymphoid follicles are covered by a specialized epithelium and are different from cryptopatches<sup>49</sup>. These structures develop postnatally in response to mucosal challenges<sup>50</sup>. Elimination of LT signalling either pharmacologically or by transfer of LT-deficient lymphocytes leads to the loss of isolated lymphoid follicles<sup>50</sup>. These findings confirm previous observations of the size of aggregates that are induced by transgenic expression of CXCL13 in the pancreas, and the potential LT dependence of the ectopic-lymphoid structures that are formed in the synovium in rheumatoid arthritis<sup>6,51</sup>.

Table 1 | Expression and regulation of lymphotoxin, LIGHT and their receptors

Molecule	Expression	Regulation	References
<b>Ligands</b>			
LT $\alpha$ /LT $\beta$	<b>Haematopoietic</b> T-, T <sub>H</sub> 1-, B- and NK-cell lineages Subset of follicular B cells T cells Lymphoid progenitor cells Inflamed sites	Activation of T, B and NK cells CXCL13 IL-4, IL-7, CCL19/CCL21 IL-7, RANKL N.D.	3,137–141 23 3,74,107 4,142 143
LIGHT	<b>Haematopoietic</b> T cells Immature DCs Granulocytes and monocytes  <b>Non-haematopoietic</b> Breast epithelial-cell line	T-cell activation N.D. N.D.  N.D.	9,144  145  146
<b>Receptors</b>			
LT $\beta$ R	<b>Haematopoietic</b> DCs and monocytes  <b>Non-haematopoietic</b> Most lineages FDCs and HEVs	N.D.  Glucocorticoids? N.D.	10,12  10,12,31,147–149
HVEM	<b>Haematopoietic</b> T cells Immature DCs, macrophages and foam cells  <b>Non-haematopoietic</b> Epithelial cells and hepatocytes	T-cell activation N.D.  N.D.	9,150 151  146,152
DCR3*	Colorectal tumours	N.D.	153

\*Only identified in humans, the existence of a mouse homologue is unlikely. CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; DC, dendritic cell; DCR3, decoy receptor 3; FDC, follicular dendritic cell; HEV, high endothelial venule; HVEM, herpes-virus entry mediator; IL, interleukin; LT, lymphotoxin; N.D., not determined; NK, natural killer; R, receptor; RANKL, receptor activator of nuclear factor- $\kappa$ B ligand.

FOLlicular DENDRITIC-CELL NETWORK (FDC network). A meshwork of specialized reticular fibroblasts that has the unique ability to retain and present intact antigen to B cells, as well as to provide specific survival and positioning signals.

CROHN'S DISEASE One of the two main forms of inflammatory bowel disease that afflicts human patients. The pathophysiology is unknown, but is presumed to stem from a dysequilibrium between the gut flora and the mucosal immune system.

Box 2 | **Lymphotoxin/LIGHT regulated aspects of the immune system****Lymphotoxin dependent***Developmental*

- **Peripheral lymph node.** Peripheral lymph-node formation<sup>4,5</sup>.
- **Mucosal system.** Mucosal lymph-node formation<sup>4,5</sup>, Peyer's-patch formation<sup>4,15</sup>, aspects of the gut lamina propria, production of IgA<sup>\*54,55</sup> and organization of the nasal-associated lymphoid tissue (NALT)<sup>\*129,130</sup>.
- **Spleen.** Organization of splenic T and B cells<sup>\*40</sup>, splenic T-cell reticulum<sup>\*40</sup> and marginal zone<sup>16,37</sup>.
- **Bone marrow.** Differentiation of natural killer (NK) cells<sup>131</sup>.

*Adult (established)*

- **Reticular networks.** All follicular dendritic-cell (FDC) networks<sup>16,29,30</sup> and splenic follicular reticulum<sup>42</sup>.
- **Germinal centres.** Formation<sup>39,72</sup> of germinal centres and positioning of cysteine-rich domain of the mannose receptor (CR-Fc)-positive DC subset<sup>132</sup>.
- **Mucosal system.** M-cell content in Peyer's patches<sup>46</sup>, Peyer's-patch and colonic-patch cellularity<sup>45</sup>, presence of colonic patches<sup>45</sup> and isolated lymphoid follicles<sup>49,50</sup>.
- **Ectopic lymphoid structures**<sup>7,133</sup>. Expression of high endothelial venule (HEV) addressin<sup>107,113</sup>.
- **Spleen.** Marginal zone<sup>136–39</sup>, and numbers and positioning of DCs<sup>27</sup>.

**LIGHT dependent**

Generation and function of cytotoxic T lymphocytes<sup>17,91</sup>, and thymic selection<sup>134</sup>.

\*Development is post-gestational in rodents. †Most probably non-developmental.

‡Observed in the context of transgenic mice and, therefore, the developmental versus established nature of this regulation is unclear. §Includes metallophilic macrophages, marginal-zone macrophages, marginal-zone B cells and the expression of mucosal addressin cell-adhesion molecule 1 (MADCAM1) by the rodent marginal-zone sinus.

Finally, a large number of lymphocytes reside in a non-organized manner in the intestinal villus, and these are phenotypically segregated into the overlaying epithelium and the inner lamina-propria compartments. The lamina propria of the intestine is home to many B cells that can secrete IgA. Early studies showed that LT-deficient mice have reduced levels of both serum and faecal IgA<sup>52,53</sup>. Not only were IgA-secreting cells absent from the gut, accounting for the loss of IgA, but also almost all B cells were absent<sup>54,55</sup>. LT is required during post-gestational development to establish a microenvironment that promotes the migration of B cells into the lamina propria, and it is in this environment that class-switching to IgA might occur<sup>54,56</sup>. As such, the general mechanisms that are invoked for FDC networks and B-cell positioning might also dictate positioning in some mucosal environments, although the specific chemokines might differ. CD157 is preferentially expressed by the reticulum in the B-cell follicle of the spleen and lymph nodes and its expression is maintained by LT $\beta$ R signalling<sup>42</sup>. Deletion of this protein led to a decreased IgA response and fewer antigen-specific antibody-forming cells in the lamina-propria compartment, indicating a link between LT-mediated regulation of potentially CD157-positive reticular elements in the lamina propria and immune function<sup>57</sup>. Overexpression of LIGHT chronically activates LT $\beta$ R and results in colitis<sup>20,21</sup>. Taken together, there is considerable evidence that

aspects of the mucosal microenvironment at all three levels — the lamina propria, the isolated lymphoid follicles and the developmentally fixed structures, such as the Peyer's patch — are affected by LT $\beta$ R signalling.

When LT has been linked to the homeostatic regulation of these various microenvironments, the LIGHT pathway has not been involved. In principle, however, LIGHT could trigger similar events and, indeed, forced expression of LIGHT can complement LT deficiency<sup>58</sup>. Such an event might have a role in reactive lymph nodes or in inflamed settings. These examples point to the general organizational roles that are played by the underlying reticular networks. Interestingly, some of the lymphoid-reticular networks seem to be relatively plastic, defying previous notions that they behave as inert scaffolds in the lymphoid organs. It is probable that the communication between mobile bone-marrow-derived cells and reticular elements during the development of lymph nodes and Peyer's patches is replayed in the maintenance of microenvironments in the secondary lymphoid system. Clearly, LT has a role in promoting and maintaining this ongoing communication.

**LT and LIGHT in immune function and disease**

The ability to manipulate some lymphoid microenvironments offers the opportunity to assess how the quality of a microenvironment affects immune function. There have been several efforts to address this topic, starting with the studies of alymphoplastic mice (*aly*), which have a defect in the ALTERNATIVE NUCLEAR FACTOR- $\kappa$ B (NF- $\kappa$ B) PATHWAY<sup>59,60</sup>. In *aly* mice, lymphoid architecture is disrupted as a result of impaired LT $\beta$ R signalling; however, it is now known that other alternative NF- $\kappa$ B signalling events that are triggered by receptors expressed by B cells and probably DCs are also disturbed<sup>61,62</sup>. In general, the analysis of various animals that are deficient in LT signalling is complicated by the absence of secondary lymphoid organs and/or defects in the development of lymphoid structure<sup>63</sup>. Similarly, linkage of the LT genes to the MHC limits experimentation to 129 and C57BL/6 backgrounds. Signalling can be eliminated in a normal animal by the use of bone-marrow chimaeras with specific gene deletions, although this experimental approach assumes that all LT expression occurs in the haematopoietic compartment. Because of these limitations, considerable use has been made of LT $\beta$ R-immunoglobulin fusion protein as a pharmacological inhibitor of LT/LIGHT signalling to probe the role of this system and microenvironments in immune function and disease in adult mice. In the following sections, we attempt to discuss B- and T-cell function independently, although in reality most of the systems represent the integrated function of both branches.

**B-cell function.** The LT system controls FDC networks and CXCR5-mediated positioning of follicular B cells (FIG. 2); however, the actual functional consequences of altered follicular environments are poorly described. Classically, in the absence of FDC networks, it would be

ALTERNATIVE NUCLEAR FACTOR- $\kappa$ B PATHWAY (NF- $\kappa$ B). Signalling through lymphotoxin- $\beta$  receptor can activate NF- $\kappa$ B through a non-canonical NF- $\kappa$ B-inducing kinase (NIK)—inhibitor of NF- $\kappa$ B kinase- $\alpha$  (IKK $\alpha$ )-dependent route that results in the activation of RelB/NF- $\kappa$ B2. The repertoire of RelB/NF- $\kappa$ B2-activated genes is presumably different from those that are activated by the classic NF- $\kappa$ B complexes.



**AFFINITY MATURATION**

The mutation of antibody variable-region genes followed by the selection of higher-affinity variants in the germinal centre leads to an increase in antibody affinity as an immune response progresses. The selection is thought to be a competitive process in which B cells compete with free antibody to capture decreasing amounts of antigen.

**SOMATIC HYPERMUTATION**

The accumulation of point mutations in the variable-region genes encoding immunoglobulin heavy and light chains, giving rise to high-affinity antibodies that are specific for a given antigen — a process known as affinity maturation. B cells that express high-affinity immunoglobulins on their cell surface are selected by limited amounts of the antigens.

expected that a loss of germinal-centre formation, impaired AFFINITY MATURATION and decreased SOMATIC HYPERMUTATION would occur. In LT-deficient mice, which lack all FDC networks, germinal-centre formation is diminished, but they do form<sup>64</sup>. These germinal centres might be similar to the short-lived T-cell-independent germinal centres that have been described previously<sup>65</sup>. In the absence of FDCs, affinity maturation using the conventional NP (4-hydroxy-3-nitrophenyl-acetyl) hapten system is largely eliminated at low doses of antigen, but is relatively unimpaired with large amounts of antigen<sup>66</sup>. At higher concentrations, antigen is presented presumably by B cells and so the requirement of FDCs is circumvented. Somatic hypermutation, which should contribute to affinity maturation, still occurs in LT-deficient animals<sup>67</sup>. More recently, somatic hypermutation was shown to occur outside the germinal centres in extrafollicular compartments, perhaps explaining the observations in LT-deficient animals<sup>68,69</sup>. An exact understanding of the role of FDC networks in germinal-centre formation and longevity, the efficiency of hypermutation, affinity maturation, and the generation of memory and plasma cells awaits more precise analyses.

How the quality of an autoreactive-antibody response impacts the development of disease is a relatively unexplored topic. Studies on the types of

autoreactive antibody specific for glycerol phosphate isomerase (GPI) that cause joint inflammation illustrate the complexity of the problem<sup>70</sup>. Similar to the GPI system, collagen-induced arthritis is another interesting model for the analysis of an autoreactive-antibody response. Mice that are immunized with type II collagen develop an autoreactive-antibody response specific for mouse collagen that triggers an immunological reaction and complement-mediated inflammation in the joints<sup>71</sup>. The model relies on contributions from both T and B cells. In this case, administration of LT $\beta$ R-immunoglobulin fusion protein was found to block the development of disease and established disease was also arrested<sup>72</sup> (TABLE 2). Treatment reduced both early- and late-phase titres of collagen-specific antibody and concomitant germinal-centre formation in the draining lymph node was found to be transient. Sustained clonal expansion of collagen-specific plasma cells was also inhibited by treatment with the fusion protein. It remains unexplored how the altered lymph-node microenvironments affect the generation and/or longevity of plasma cells, and exactly which aspects of the collagen-specific response were altered. At present, LIGHT has not been linked to B-cell function.

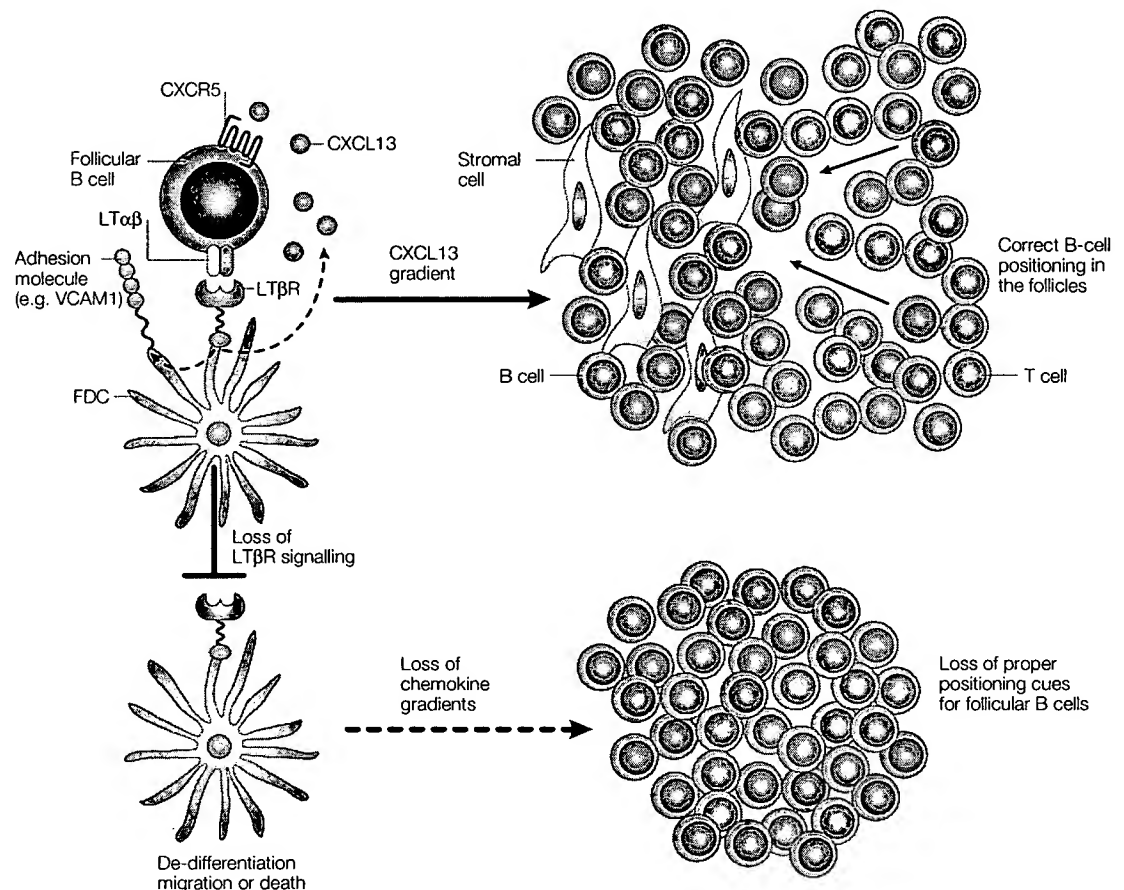
**T-cell function.** Probably the first clue that the LT pathway is involved in T-cell function was the observation that expression of RNA encoding LT $\alpha$  is a marker of the differentiation of T cells into the pro-inflammatory T helper 1 (T<sub>H</sub>1) subtype<sup>73,74</sup>. Later, administration of the LT/LIGHT inhibitor — LT $\beta$ R-immunoglobulin fusion protein — was found to ameliorate disease in two T-cell-based mouse models of colitis<sup>75</sup>. In the CD45RB<sup>hi</sup> model, CD4<sup>+</sup> T cells that are depleted of CD45RB<sup>low</sup> regulatory cells are transferred to immunodeficient recipients, in which they aberrantly colonize the gut. A second, conceptually similar, T-cell-transfer model produced similar results (TABLE 2). In both cases, treatment with LT $\beta$ R-immunoglobulin fusion protein prevented the development of disease and reduced inflammation in the colon<sup>75</sup>. Given the crucial role for T cells in these transfer models, these results indicate that inhibition of the LT/LIGHT pathway affects T-cell function. Subsequent work has evaluated the effect of LT/LIGHT inhibition in many other T-cell-dependent immunological settings and this work is summarized in TABLE 2.

Challenge with virus is one method for assessing CD8<sup>+</sup> T-cell responses and several studies have addressed this aspect. LT-deficient mice have an impaired ability to clear infection with lymphocytic choriomeningitis virus (LCMV), mouse cytomegalovirus (CMV), herpes simplex virus (HSV) and mouse  $\gamma$ -herpes virus, and have delayed responses to infection with influenza<sup>76–82</sup>. Host-defence responses vary widely with different viruses, although CD8<sup>+</sup> T-cell responses have been examined in the case of infection with LCMV and HSV. It is important to distinguish whether LT is directly required for the CD8<sup>+</sup> T-cell response or whether diminished antiviral activity is a consequence of altered lymphoid architecture that is caused by LT inhibition, especially

**Box 3 | Reticular stromal networks in lymphoid organs**

Lymphoid organs contain reticular networks that function to provide anchoring and/or positioning points for some cell types. Originally believed to be relatively inert scaffolds, there is the increasing recognition that these scaffolds are specifically regulated and provide support signals to cells that are undergoing specialized events — for example, in the germinal centres. These networks seem to be composed mainly of fibroblastoid cells or as cell layers that coat collagen fibres. Several such networks have been identified in the spleen and lymph nodes.

- **B-cell follicle.** There is a general reticular network in primary B-cell follicles that can be stained by antibodies specific for CD157 (REF. 57). It is suspected that this network is more extensive than the follicular dendritic-cell (FDC) network and might be a less mature FDC network<sup>22</sup>. The FDC is a specialized fibroblastoid cell that forms a network in the primary follicle. After formation of the germinal centre, this network condenses and matures. Differentiation of the germinal centre FDC network in mice is noted by the expression of FDC-M1 antigen and increased expression of vascular-cell adhesion molecule 1 (VCAM1)<sup>32</sup>.
- **T-cell zone.** The network in the T-cell zone of the periarteriolar lymphocyte sheath (PALS) differs from that of the B-cell follicle and is distinguished by an antibody specific for gp38 (REF. 40). In contrast to the FDC network, relatively little is known about this network<sup>135</sup>. In the lymph node, these cells form a reticular network in the proximity of high endothelial venules and presumably provide a meeting place to enhance the encounter of antigen-presenting cells (APCs) with T cells.
- **Marginal zone.** There is a reticular network in the splenic marginal zone that can be identified by ERTR7 staining. This network might function to provide the intercellular adhesion molecule 1 (ICAM1) and VCAM1 interactions, which are required to retain the marginal-zone B cells in the compartment.
- **Medullary zone.** The scaffold elements in this region form the medullary cords that presumably function to organize cellular trafficking out of the lymph node, as well as being potentially involved in plasma B-cell biology<sup>136</sup>. It is probable that similar elements exist around the bridging channels and red pulp of the spleen. This aspect is relatively unexplored.



**Figure 2 | Maintenance of follicular dendritic cells — a model for lymphotoxin–stromal-cell interactions.** Activated lymphocytes, as well as a subset of resting B cells, express lymphotoxin  $\alpha\beta$  (LT $\alpha\beta$ ) heteromers on their cell surface. LT expressed by lymphocytes signals to stromal cells, which express the LT $\beta$  receptor (LT $\beta$ R). Constitutive signalling through the LT $\beta$ R maintains stromal cells in a differentiated state. For example, expression of follicular-dendritic-cell (FDC) markers — CD35, FDC-M1, FDC-M2, vascular-cell adhesion molecule 1 (VCAM1) (in primates) and mucosal addressin cell-adhesion molecule 1 (MADCAM1) — is lost from the spleen after treatment with inhibitors of the LT pathway<sup>29,30</sup>. In mice, these markers disappear within 1–3 days of treatment, indicating that the LT $\beta$ R signal needs to be continuous, and highlights the highly dynamic and plastic behaviour of this reticular network. LT-maintained FDCs, in turn, secrete the CXC chemokine ligand 13 (CXCL13), which recruits CXCR5-positive B cells and T helper cells into the follicle<sup>22,106</sup>. CXCL13 can induce the expression of LT in a transient manner by follicular B cells, forming a feedback loop that maintains cellular positioning and polarization of the follicle<sup>23</sup>. The expression of LT by cells in the germinal centre does not depend on CXCL13, and it is possible that CD40 signalling induces the expression of LT by germinal-centre B cells<sup>23</sup>. In an analogous manner, induction of LT expression by T cells in response to CCR7 signalling has been shown<sup>107</sup>. Amazingly, simple ectopic non-lymphoid expression of CXCL13 is sufficient to instruct the organization of T and B cells into a lymph node-like structure<sup>107</sup>, indicating that B-cell recruitment encourages the subsequent recruitment of T cells. In the follicles, CXCL13 is produced mainly by stromal reticular elements, but dendritic cells and macrophages can produce this chemokine and this source is important in other settings — for example, in the peritoneal cavity and in autoimmune disease<sup>108–110</sup>.

in the case of LCMV. Indeed, two studies have clearly shown that the microarchitecture of the spleen contributes to this effect and that LT $\alpha$ -deficient CD8<sup>+</sup> T cells can respond normally<sup>76,81</sup>. By contrast, LT $\beta$ R–immunoglobulin fusion protein rescued NZB mice from LCMV-induced shock, and other than decreased FDC networks, it was unlikely that changes in the microarchitecture occurred within the short time frame of the LCMV-infection protocol<sup>83</sup>. Therefore, the LT/LIGHT pathway probably has a more direct role in CD8<sup>+</sup> T-cell responses. Consistent with this point, rejection of transplanted allogeneic intestine was prevented by administration of LT $\beta$ R–immunoglobulin fusion protein in a setting in which the rejection mechanism was restricted to CD8<sup>+</sup> T cells<sup>84</sup>.

T cells are important in the pathogenesis of diabetes, and the effect of inhibition of the LT/LIGHT pathway on insulin-dependent diabetes mellitus was examined using NON-OBESE DIABETIC (NOD) MICE. Mice that express a neutralizing LT $\beta$ R–immunoglobulin chimeric transgene were backcrossed onto the NOD background<sup>37</sup>. Development of disease, *ex vivo* T-cell proliferation and secretion of interferon- $\gamma$  (IFN- $\gamma$ ) in response to glutamic acid decarboxylase (GAD) antigen were attenuated in fusion-protein-expressing mice. Similarly, treatment with LT $\beta$ R–immunoglobulin fusion protein prevented the development of disease at a relatively late stage<sup>85</sup>. Why these T cells were sensitive to LT $\beta$ R–immunoglobulin fusion-protein treatment remains unresolved in this system.

**NON-OBESE DIABETIC MICE (NOD mice).** A strain of mice that normally develop idiopathic autoimmune diabetes that closely resembles type I diabetes in humans. The target antigen(s) that is recognized by the pathogenic CD4<sup>+</sup> T cells that initiate disease is expressed by pancreatic-islet cells, but its identity has remained elusive.

Table 2 | The effects of inhibition of the lymphotoxin/LIGHT pathway in rodent disease models

Human disease	Model	Means of pathway inhibition	References
<b>Inhibition of disease</b>			
Arthritis	Collagen-induced arthritis <sup>**</sup>	LT $\beta$ R-Ig fusion protein	72
	Adjuvant-induced arthritis <sup>‡</sup>	LT $\beta$ R-Ig fusion protein	72
Inflammatory bowel disease	CD45RB <sup>hi</sup> colitis	LT $\beta$ R-Ig fusion protein	75
	CD3e-transgenic colitis	LT $\beta$ R-Ig fusion protein	75
	T <sub>H</sub> 2-type TNBS-induced colitis	LT $\beta$ R-Ig fusion protein	45
Multiple sclerosis	Rat acute EAE <sup>‡</sup>	LT $\beta$ R-Ig fusion protein and LT $\beta$ -specific antibody	88
	Relapsing/remitting EAE <sup>‡</sup> in SJL mice	LT $\beta$ R-Ig fusion protein	88
Myasthenia gravis	Experimental autoimmune myasthenia gravis <sup>‡</sup>	LT $\alpha$ deficiency, LT $\beta$ deficiency	154
Insulin-dependent diabetes	Non-obese diabetic mice <sup>*</sup>	LT $\beta$ R-Ig fusion protein and LT $\beta$ R-Ig transgene	37,85
Transplantation	Intestine (CD8 <sup>+</sup> T-cell-mediated rejection)	LT $\beta$ R-Ig fusion protein and LT $\beta$ -specific antibody	84
	Acute graft-versus-host disease	LT $\beta$ R-Ig fusion protein and LIGHT deficiency	90
	Heart	LIGHT deficiency	19
Virus shock syndrome	LCMV-infected NZB mice <sup>*</sup>	LT $\beta$ R-Ig fusion protein	83
<b>No effect</b>			
Arthritis	Collagen monoclonal antibody/LPS-induced acute arthritis	LT $\beta$ R-Ig fusion protein	72
Inflammatory bowel disease	T <sub>H</sub> 1-type TNBS-induced colitis	LT $\beta$ R-Ig fusion protein	45
Multiple sclerosis	MOG and pertussis toxin-induced EAE	LT $\beta$ R-Ig fusion protein and LT $\alpha$ deficiency	86-88,155 <sup>§</sup>

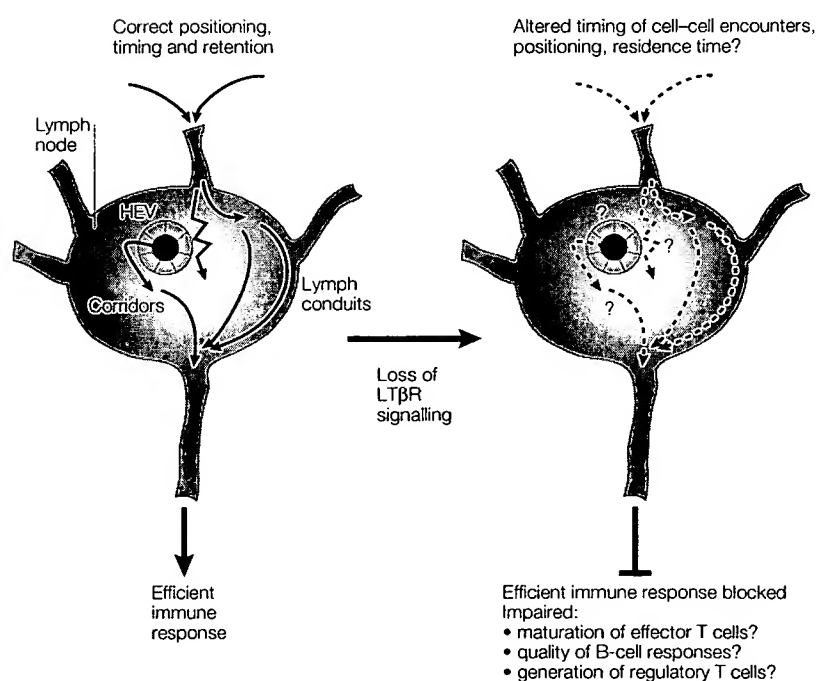
<sup>\*</sup>Efficacy with established disease, otherwise, all data represent prophylactic conditions. <sup>‡</sup>This model requires administration of *Mycobacterium tuberculosis* in the form of complete Freund's adjuvant. <sup>§</sup>Suen *et al.* showed a main role for lymphotoxin- $\alpha$  (LT $\alpha$ ) without major involvement of LT $\beta$ . EAE, experimental autoimmune encephalomyelitis; LCMV, lymphocytic choriomeningitis virus; LPS, lipopolysaccharide; LT $\beta$ R-Ig, lymphotoxin- $\beta$  receptor-immunoglobulin fusion protein; MOG, myelin oligodendrocyte glycoprotein; TNBS, trinitrobenzene sulphonate acid.

To evaluate further the role of LT/LIGHT pathway in T-cell-mediated disease, we have studied three separate models of experimental autoimmune encephalomyelitis (EAE). In previous studies with LT-deficient mice, LT $\alpha$  and to a lesser extent LT $\beta$  were shown to be required for the development of EAE; however, in a separate study, EAE was shown to progress normally in chimaeric mice with LT $\alpha$ -deficient lymphocytes<sup>86,87</sup>. We re-visited the role of LT in EAE using LT $\beta$ R-immunoglobulin fusion protein to inhibit the LT pathway in adult animals<sup>88</sup>. Inhibition of the LT/LIGHT pathway resulted in the prevention of disease in an acute rat model, and relapses in a chronic relapsing–remitting mouse model. It is notable that direct depletion of activated T cells by LT $\beta$ R-immunoglobulin fusion protein does not contribute to the mode of action<sup>72,88</sup>. Treatment with LT $\beta$ R-immunoglobulin fusion protein had no effect on myelin oligodendrocyte glycoprotein (MOG)-induced EAE, which, unlike the other two models, requires the administration of pertussis toxin. In addition, the original studies on gene-deficient animals were carried out using the same pertussis toxin-dependent model. Pertussis toxin inhibits signalling through G-protein-coupled chemokine receptors. Given that expression of some chemokines in the secondary lymphoid tissues is at least under partial control of LT and, moreover, the chemokine-mediated induction of cell-surface expression

of LT by B cells is blocked by treatment with pertussis toxin, this approach to evaluate the role of LT in EAE might have obscured its biological function<sup>23</sup>.

Pharmacological studies that use LT $\beta$ R-immunoglobulin fusion protein cannot distinguish between inhibition of the LT–LT $\beta$ R interaction and LIGHT binding to either LT $\beta$ R or HVEM. Early *in vitro* work indicated that LIGHT has a role in T-cell co-stimulation and many *in vivo* studies have now firmly linked LIGHT to T-cell function<sup>9</sup>. LT $\beta$ R-immunoglobulin fusion protein was effective in reducing acute graft-versus-host disease and this was attributed to the inhibition of LIGHT<sup>89,90</sup>. This approach was especially effective when combined with blocking of CD40 (REF. 90). Although LIGHT-deficient animals can reject cardiac allografts, rejection could be prevented by treatment with cyclosporin in LIGHT-deficient animals, but not in the control littermates<sup>19</sup>. Some aspects of CD8<sup>+</sup> T-cell function seem to require LIGHT, as the generation of cytotoxic T lymphocytes (CTLs) or responses to superantigen were impaired in LIGHT-deficient cells<sup>17,91</sup>. Finally, LIGHT-transgenic mice develop severe lymphadenopathy and T-cell-mediated autoimmune disease and succumb to colitis within 6 months<sup>20,21</sup>. Therefore, it is probable that some of the efficacy of LT $\beta$ R-immunoglobulin fusion protein derives from the inhibition of LIGHT-mediated activation of either LT $\beta$ R or HVEM. However, not all the activity of

LT $\beta$ R-immunoglobulin fusion protein can be attributed to the inhibition of LIGHT. Two studies have indicated a clear contribution of LT-LT $\beta$ R signalling to T-cell function. In the case of CD8 $^{+}$  T-cell-mediated rejection of an intestinal transplant, Guo *et al.*<sup>84</sup> showed that a blocking monoclonal antibody specific for LT $\beta$  could prevent rejection. Similarly, in a rat EAE model, a blocking LT $\beta$ -specific monoclonal antibody prevented EAE, whereas an HVEM-immunoglobulin fusion protein had no effect<sup>88</sup>. So, binding of LT to LT $\beta$ R-immunoglobulin fusion protein is important for the development of disease, at least in some T-cell-dependent systems. It might be useful to hypothesize that LT affects T-cell function through effects on lymphoid microenvironments, whereas the effects of LIGHT focus on co-stimulatory activity, in particular aspects involving CD8 $^{+}$  T cells.



**Figure 3 | What is the role of lymphotoxin/LIGHT in T-cell function in the lymph nodes?**

A schematic diagram of the lymph and cellular flow through a lymph node. Inhibition of the lymphotoxin (LT)/LIGHT pathway is surprisingly effective in many T-cell-based models of autoimmune disease and much of this biology occurs in the lymph node. The essential aspects of lymph-node microenvironments that are LT/LIGHT dependent remain unclear, especially in a reactive lymph node after exposure to adjuvant. The dotted arrows illustrate potential areas in which LT/LIGHT-dependent phenomena might occur — that is, trafficking through the high endothelial venules (HEV), trafficking of antigen-expressing cells that come from the lymph, retention of activated T cells and the longevity of T-cell-dendritic-cell (DC) complexes, for example. T cells enter through the HEV gate and remain in regions that are proximal to the HEV, known as 'corridors'<sup>111,112</sup>. Treatment with LT $\beta$  receptor (LT $\beta$ R)-immunoglobulin fusion protein reduced the expression of peripheral lymph-node addressin and mucosal addressin cell-adhesion molecule 1 (MADCAM1) by HEVs in established ectopic lymphoid structures in the pancreas<sup>107</sup>. Similarly, transgenic expression of LT $\alpha$  could induce the sulphotransferase that is required for the expression of the ligand for L-selectin on the luminal face of HEVs in ectopic lymphoid structures<sup>113</sup>. Therefore, it is possible that LT can regulate aspects of HEV maturation. HEV are known to be rather plastic, as their maturation status depends on the flow of afferent lymph<sup>114</sup>. Once in the corridors, lymphocytes must gain contact with antigen-expressing DCs. The lymph node has a highly complex architecture with systems of conduits that channel lymph through the node. Paradoxically, however, lymph from the tissue bed seems to remain in these specialized conduits and only low-molecular-weight proteins gain direct access to the T-cell compartment<sup>115</sup>. How soluble antigens and antigen-presenting cells in the lymph gain access to the T-cell compartment remains relatively unexplored. In contrast to the T-cell compartment, lymph might gain direct access to the B-cell follicles<sup>112</sup>.

Which aspects of T-cell biology are LT dependent? In both chronic relapsing-remitting diseased mice and acute rat EAE models, lymphocyte migration to and within the parenchyma of the central nervous system was impaired by treatment with LT $\beta$ R-immunoglobulin fusion protein. So, T-cell migration might be LT or LIGHT dependent. The CXCR3 ligands — CXCL9, CXCL10 and CXCL11 — are crucial for the migration of effector cells to inflamed tissues. In studies of intestinal transplant rejection, inhibition of LT led to reduced levels of expression of CXCL9 by the graft, providing some *in vivo* support for such a potential mechanism<sup>84</sup>. Moreover, we have found that LT $\beta$ R signalling results in the production of these CXCR3 ligands both *in vitro* and *in vivo* (M. Lukashev and J. L. B., unpublished observations).

In addition to T-cell migration, there is also evidence that treatment with LT $\beta$ R-immunoglobulin fusion protein impairs T-cell function. In the EAE studies, we found that CD4 $^{+}$  T cells derived from LT $\beta$ R-immunoglobulin fusion protein treated rats or mice were hyporesponsive to myelin basic protein (MBP) and proteolipid protein (PLP) peptides, respectively, and secretion of IFN- $\gamma$  was also reduced. It is unlikely that the impaired T-cell recall responses indicate defects in the early priming of T cells for two reasons: first, only relapses and not the acute phase of relapsing-remitting EAE are prevented by administration of LT $\beta$ R-immunoglobulin fusion protein. Second, using an adoptive transfer T-cell-transgenic system, initial clonal expansion of CD4 $^{+}$  T cells *in vivo* was found to be unaffected by inhibition of LT/LIGHT, although recall responses *ex vivo* were impaired. These results are paralleled by studies on CD8 $^{+}$  T-cell responses to HSV. Specifically, LT $\alpha$ -deficient mice that are infected with HSV had normal numbers of virus-specific CD8 $^{+}$  T cells, yet these cells were not IFN- $\gamma$  positive and retained high expression levels of the naive T-cell marker L-selectin<sup>80</sup>. Therefore, clonal expansion of CD8 $^{+}$  T cells can occur in the altered microarchitecture of LT $\alpha$ -deficient mice, but these cells do not mature into proper effector cells. Our studies<sup>88</sup> and the studies of Berger *et al.*<sup>82</sup> indicate that inhibition of the LT pathway during the initiation of an immune response might have effects on CD4 $^{+}$  or CD8 $^{+}$  T cells that are only manifested later in the immune response. One possible explanation for the phenomenon of late-stage T-cell suppression is that inhibition of the LT pathway could provoke the generation of T regulatory cells, and this possibility is under investigation at present.

It will be important to determine whether T-cell hyporesponsiveness is due to a direct or indirect effect of LT inhibition. In the case of a direct effect, activated lymphocytes that express LT might trigger LT $\beta$ R, which has been shown to be expressed by DCs, and this triggering could induce the maturation of DCs or the secretion of cytokines<sup>10,12</sup>. Indeed, it has been shown that DCs isolated from LT $\beta$ -deficient mice that have been immunized with LCMV secrete less IL-12 (REF. 82). We favour the hypothesis that the effects of the LT pathway

on T cells might be indirect and are a consequence of an altered lymphoid microenvironment in the draining lymph node. Relevant to this point is the fact that many of the experimental models of autoimmune disease that have been discussed here depend on immunization with autoantigen in the presence of a strong mycobacterium adjuvant (TABLE 2). It is probable that one function of the mycobacterium-containing adjuvant is to establish a favourable microenvironment in the draining lymph node, and there is suspicion that aspects of this process are affected by treatment with LT $\beta$ R-immunoglobulin fusion protein<sup>72</sup>. The development of disease in all autoimmune disease models that use immunization in this manner are inhibited by LT $\beta$ R-immunoglobulin fusion protein (except where pertussis toxin was co-administered). For example, complete Freund's adjuvant alone will induce joint inflammation in the autoimmune-prone Lewis rat and this event was blocked by inhibition of the LT/LIGHT pathway<sup>72</sup>. So, the LT/LIGHT pathway might affect the ability of mycobacteria to establish a favourable microenvironment in the inflamed draining lymph node and, although these events might be inconsequential for early clonal expansion of T cells, they could have an impact on late-phase T-cell effector responses.

#### The frontier: lymph-node microenvironments

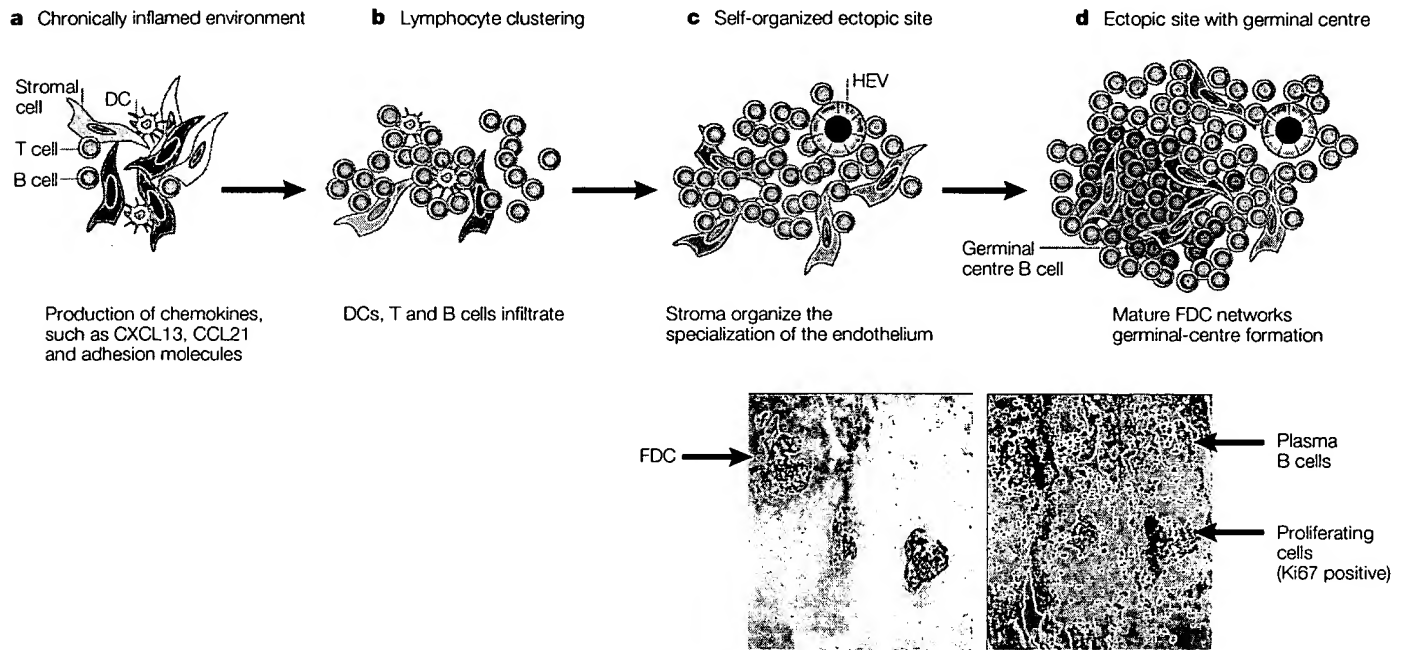
It is now established that some important aspects of T-cell function depend on the LT/LIGHT pathway and, in two settings, LT itself has been shown to be crucial. Much of this biology occurs in the draining lymph node and, in contrast to the spleen, it remains unclear exactly which aspects of the lymph-node microenvironment are LT dependent. There are hints that LT/LIGHT inhibition results in aberrant lymph-node trafficking events. For example, LT $\beta$ R-immunoglobulin fusion protein decreases the cellularity in Peyer's patches and can reduce the marked expansion of T- and B-cell numbers in a lymph node that drains an inflamed site — for example, after subcutaneous injection of adjuvant<sup>72</sup> (R.F. Fava and J. L. B., unpublished observations). Increased numbers of antigen-specific cells are unlikely to account for the marked increase, indicating that LT/LIGHT modulates the entry or retention of cells in the reactive lymph node. Additionally, much of the organization of lymph-node compartments involves a highly specialized vascular endothelium, reticular fibroblasts and fibroblast-wrapped collagen cords (FIG. 3). Aspects of these microenvironments are potential candidates for being controlled by LT. After contact with DCs, T cells become activated and the nature of these encounters — that is, their duration, contact with mature versus immature DCs and the nature of the stromal environment — probably influences the response both qualitatively and quantitatively. It is in this situation that LT/LIGHT inhibition might have a role. Understanding the details of T-cell activation in this complex architecture is an important new frontier for immunology. Given the use of LT inhibitors in dissecting the role of the FDC network in B-cell responses, similar advantage might be achieved with respect to T-cell-lymph-node interactions.

#### Therapeutic potential

Biological immunosuppressive strategies that are available at present mainly involve the inhibition of pro-inflammatory mediators, such as TNF or chemokines; co-stimulatory events, for example, CD28 or CD40 signalling; the depletion of crucial cellular components, such as B cells or memory T cells; and the inhibition of integrin-based trafficking events. The case of LT/LIGHT inhibition is unique, as alterations in several lymphoid microenvironments are expected with consequences for both the T- and B-cell branches of the immune system. In autoimmune disease, both innate and adaptive aspects of the pathological response need to be normalized and, finally, the system must be subdued by generating tolerance to the various autoantigens that perpetuate the disease. Only then will the disease be 'cured'. Several avenues to tolerance have been explored, but the matter of altering the timing, strength and duration of antigen-specific-receptor signalling encompasses many of these strategies. In effect, the microenvironment is a main determinant of an efficient response and altered timing or positioning of the cellular components might provide another route to the same end. Induction of tolerance by LT/LIGHT inhibition has not been formally shown, but the lack of relapses in one EAE model is suggestive.

The LT $\beta$ R-immunoglobulin fusion protein itself is promising, not only because of its ability to modulate microenvironments, but also because it is a dual pathway inhibitor that affects both LIGHT- and LT-induced events. On the basis of the rodent models, type I diabetes, colitis, multiple sclerosis, rheumatoid and psoriatic arthritis and aspects of organ rejection might be positively influenced by treatment with LT $\beta$ R-immunoglobulin fusion protein. TNF inhibitors have proven to be an important advance in the treatment of arthritis, psoriasis and Crohn's disease. Given the ability of LT $\beta$ R-immunoglobulin fusion protein to modulate intestinal function at three different levels and the colitis that is induced by transgenic expression of LIGHT in T cells, human colitis might be an appropriate target for therapy based on LT/LIGHT inhibition. Despite the vestigial TNF $\beta$  nomenclature for LT $\alpha$ , the TNF and LT pathways are fundamentally distinct. A good example of the difference between the TNF and LT pathways is illustrated in the case of EAE. Administration of antibody specific for TNF ameliorates EAE in the early stages, but in the late stages, enhanced T-cell responses are observed in rodents, as well as disease exacerbation in clinical trials of multiple sclerosis<sup>92,93</sup>. This pattern contrasts with LT $\beta$ R-immunoglobulin fusion-protein treatment of chronic EAE, in which late-stage relapses rather than the early acute-phase disease are inhibited. Therefore, LT-directed therapies might offer advantages in the treatment of patients that are resistant to TNF inhibitors or as an addition to TNF therapy in other settings.

As many autoimmune diseases are accompanied by the formation of pathological ectopic-lymphoid structures, LT-based therapy has additional potential (FIG. 4). Some of these diseases might be markedly exacerbated



**Figure 4 | Organized lymphoid tissue is seen in inflamed ectopic sites.** Chronic inflammation can trigger the formation of lymphoid aggregates in the inflamed sites. Lymphocytes that express lymphotoxin (LT), or possibly LIGHT, can induce stromal cells to release chemokines — for example, CXC chemokine ligand 13 (CXCL13) — that then attracts CXC chemokine receptor 5 (CXCR5)-positive B cells. Experimentally, ectopic expression of CXCL13, CCL21 and LT can induce the formation of such aggregates<sup>107,113,116,117</sup>. Apparently, attraction of either the B cells with CXCL13 or the T cells and dendritic cells (DCs) with CCL21 is sufficient to nucleate the structure and then the other cell types will self-organize. T- and B-cell-rich zones emerge, DCs enter, high endothelial venules (HEVs) and follicular DC (FDC) networks form and germinal centres develop. In human tissues, such organized aggregates have been noted in Crohn's disease, rheumatoid arthritis, Sjögren's syndrome, autoimmune thyroiditis, myasthenia gravis, chronic bacterial infection, atherosclerotic plaques and chronic inflammatory liver disease<sup>94,118</sup>. The inserted image shows serial sections of an organized lymphoid structure in the synovium of a patient with rheumatoid arthritis stained for FDC networks (left) and plasma cells and proliferating Ki67-positive B cells in the germinal centres (right). Image reproduced with permission from REF. 119 © American Association of Immunologists (1999). Expression of CXCL13 and CCL21 is often observed in ectopic lymphoid structures in humans<sup>94</sup>.

by local organization that improves the efficiency of autoreactive responses. As it is probable that LT $\beta$ R signalling underlies some of this ectopic organization, its inhibition could be directly beneficial<sup>94</sup>. Local microenvironments are crucial in other settings. For example, follicular lymphomas and lymphomas of the mucosa-associated lymphoid tissue (MALT) might require nurturing microenvironments, and removal of FDC networks could lead to the loss of chemokine positioning signals and elimination of the 'survival niche'<sup>95</sup>. FDC networks are crucial in the initial stages for prion infectivity and, therefore, accidental exposures could be treated prophylactically<sup>96,97</sup>. FDC networks harbour HIV particles and seem to be involved in the generation of highly infectious virus<sup>98</sup>. Elimination of this reservoir could have advantages at the beginning of antiviral therapy. Last, as immunologists begin to comprehend the role of the splenic marginal zone and the mucosal IgA-producing environments in pathology, the ability to modulate these compartments might become useful. In addition, the splenic marginal zone is a relatively large compartment in humans and so its potential contribution to immunoglobulin-based autoimmune disease might be unappreciated.

### Concluding remarks

Now, approximately 20 years after the molecular description of LT $\alpha$ , the fundamental biology of this system has taken shape. The combination of a biochemically challenging heteromeric LT ligand, the many receptors and ligands and the developmental defects that are associated with their deletion have conspired to make functional analyses difficult. A picture is emerging in which the reticular stromal cells in the immune system have considerable control over the local microenvironments. Perturbation of these microenvironments might render the immune system 'out-of-tune' with repercussions at many levels. As the genes encoding TNF and LT are closely linked, it is perhaps not surprising that the biology of the LT system now seems as rich and vital as that of TNF. The next frontier in understanding the role of these microenvironments is the formation of a complete picture of how cells traffic through the various secondary lymphoid organs and how progression is controlled both temporally and spatially. Only then will we begin to understand how the microenvironment influences the outcome of the various cellular encounters that lead to an efficient immune response.



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#### Acknowledgements

We wish to thank E. Notidis and P. Hochman for critical reading and C. Ware, P. Rennert, Y.-Z. Fu and J. Cyster for many helpful discussions.

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